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# Riverside wren pairs jointly defend their territories against simulated intruders

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**Abstract**

Duets are a jointly-produced signal where two or more individuals coordinate their vocalizations by overlapping or alternating their songs. Duets are used in a wide array of contexts within partnerships, ranging from territory defence to pair bond maintenance. It has been proposed that pairs that coordinate their songs might also better coordinate other activities, including nest building, parental care, and defending shared resources. Here, we test in the riverside wren (*Cantorchilus semibadius*), a neotropical duetting species that produces highly coordinated duet songs, whether males and females show similar responses to playback. During territorial disputes in songbird species, individuals tend to direct their attention towards same-sex territorial intruders, but this bias might be less pronounced in duetting species. We performed a dual-speaker playback experiment to examine how mated individuals respond to speakers broadcasting female versus male duet contributions. We found that riverside wrens have high levels of converging behaviour by duetting and remaining in close proximity of one another when responding to simulated paired intruders. Males and females spent more than 80% of their time less than one meter apart while defending their territory. Both individuals in a pair aggressively engaged with both male and female simulated trespassers by approaching equally close and spending equal time near the two speakers. These results suggest that both sexes perceive a paired territorial intrusion as a similar threat and that both partners are highly invested in defending the shared resources. The current study is one of the few to demonstrate equal attention and aggression from mated pairs towards simulated same-sex and opposite-sex intruders. We suggest that pairs responding together, in close proximity of one another, might be favorable in duetting species when defending the territory because maintaining a close distance between partners facilitates the extreme coordination of their joint territorial signals.

*Keywords*: vocal duets, territorial defence, Riverside wren, *Cantorchilus semibadius*,

cooperation.

## Introduction

The exchange of acoustic signals between individuals is a crucial component of many aspects of animal behaviour including mate attraction, territory defence, parent-offspring communication, and species recognition (Kroodsma & Miller, 1982; Searcy & Anderson, 1986; Catchpole & Slater, 2008). The information transmitted through these signals depends on the signaler's and receiver's social and spatial relations (Naguib, 2005). For instance, during territorial defence in songbird species, individuals must choose whether to avoid, tolerate, or fight intruders depending on the vocal interactions between all participants (Tanner & Adler, 2009). Territorial disputes become more complex in species where both sexes participate in defending the shared resources. For example, the majority of studies done so far have found that females and males mostly direct their attention and aggression towards same-sex intruders (Slagsvold, 1993; Levin, 1996b; Seddon, Butchart, & Odling-Smee, 2002; Grafe & Bitz, 2004; Mennill 2006; Rogers, Langmore, & Mulder, 2007, Cain, Ainsworth, & Ketterson, 2011). However, this sex-specific bias is not as consistent in species where both partners vocalize together (Hall & Peters, 2008; Mennill & Vehrencamp, 2008; Illes & Yunes-Jimenez, 2009; Templeton, Rivera-Cáceres, Mann, & Slater, 2011), suggesting that the coordination required for the vocal behaviour might be facilitated by performing a joint defence and maintaining a close distance between partners. In a meta-analysis performed by Logue (2005) to test if duetting species showed a significant reduction in the sex-specific territorial defence behaviours, he found that a cooperative territorial defence was indeed more common in duetting than non-duetting birds.

Duets are mainly regarded as coordinated displays where individuals alternate or overlap

78 their vocalizations to achieve an outcome beneficial to both partners (Hall, 2009). Duets
79 signal the stability of the partnership to territorial rivals (Hall, 2000; Mann, Marshall-Ball, &
80 Slater, 2003; Hall & Magrath, 2007), enhance acoustic contact and pair bonding (Logue &
81 Gammon, 2004; Mennill & Vehrencamp, 2008), and they might have a role in achieving
82 reproductive synchrony (Hall, 2009). Duets are often performed in counter-singing
83 interactions with neighbouring pairs and are frequently produced in response to territorial
84 intrusions (Logue, 2005; Mennill & Vehrencamp, 2008; Hall, 2009). As avian duets are
85 usually a multifunction joint signal, different species have been shown to use their
86 coordinated vocalizations in different manners to solve territorial disputes (Hall, 2009;
87 Douglas & Mennill, 2010; Dahlin & Benedict, 2014). There are two main ways in which duets
88 seem to function cooperatively against intruders: through division of labour, where each
89 member defends their territory and partner from the same-sex intruder (Levin, 1996b;
90 Mennill, 2006; Mennill & Vehrencamp, 2008; Templeton et al. 2011), or by presenting a
91 stronger unified front against trespassers (Hall, 2000; Hall & Peters, 2008; Dahlin & Wright,
92 2012). In species that have sexes varying in weight, individuals might stay with the same-
93 sex intruder to avoid the risks of interacting with bigger birds (Logue & Gammon, 2004;
94 Marshall-Ball, Mann, & Slater, 2006). However, in species that perform duets with a fine-
95 scale temporal coordination, it has been suggested that singing highly coordinated duets
96 when defending a territory could signal a strong commitment within the pair and hence, a
97 strong motivation to defend the territory (Hall, 2000; Marshall-Ball et al., 2006; Hall &
98 Magrath, 2007; Logue, 2007). Because temporal coordination within duets is higher when
99 pairs are closer together (Mennill & Vehrencamp, 2008; Templeton et al., 2013a), birds risk
100 losing that precision if they split up and perform a same-sex defence strategy. If singing with
101 temporal precision is an advantage when facing intruders then it seems likely that duetting
102 pairs will try to maintain that precision by staying together.
103

In this study we investigated the degree of vocal duetting and the physical responses of riverside wrens, *Cantorchilus semibadius*, during territory defence. Riverside wrens sing some of the most complex and highly precise antiphonal duets (Mann, Dingess, Baker, Graves, & Slater, 2009). Despite the fact that partners reply immediately to one another (on average after 0.06 – 0.01 s), vocalizations rarely overlap (Mann et al., 2009). Riverside wrens are socially monogamous and pairs have year-round territories (Skutch, 2001). Both sexes perform solo songs and contribute to duets by selecting from a sex-specific repertoire, and it has been estimated that individuals of each sex possess as much as 40 phrase types in their repertoires (Walters, 2013). When performing duets, the pair follows a duet code (Logue, 2006), resulting in one or both individuals selecting a particular phrase type according to its partner's choice. We used a stereo-duet playback design to study the interactions within pairs and to disentangle the interactions between each bird and same-sex and opposite-sex intruders (speakers). Due to the highly precise acoustic coordination this species shows, we predicted that individuals would follow a joint defence strategy (Seibt & Wickler, 1977) rather than a division of labour strategy. We predicted males and females would respond together and stay in close proximity instead of splitting up spatially with males interacting mainly with the male intruder and females interacting mainly with the female intruder.

## Methods

### Field methods

We studied riverside wrens at Osa Conservation's Piro field station in Costa Rica. The station is in lowland and wet rainforest on the Osa Peninsula in southern Costa Rica (8°24'6.96" N, 83°20'10.74" W). Riverside wrens are common at the study site, especially

next to rivers and wetlands. Riverside wrens nest throughout the year and remain with their offspring for up to five months (Skutch, 2001). We have studied this population of riverside wrens since 2013, and we have colour banded and collected biometric data from more than 100 individuals at the study site. Riverside wrens' territories have an average of 0.61 ± 0.04 ha (unpublished data). To estimate the size of the territories we followed the focal pairs during the recordings and we mapped their boundaries with a hand-held global positioning system (Garmin GPS-60SCx, Garmin, Olathe, KS, USA). Adult and juveniles were captured with mist nets and banded with a unique combination of leg bands, including three coloured plastic and one numbered metal band, for individual identification. We measured each bird's weight, wing length, and tail length upon capture. We distinguished juveniles from adults by the colour of the bill (yellow underside of bill in juveniles, dark bill in adults) and the colour of the eye (grey iris in juveniles, brown iris in adults). We distinguished females from males by the presence of a brood patch (if present), by their songs (see Fig. 1) and, if both adults were captured, also by the relative body measurements since sexes are moderately dimorphic. Males in the population (n = 51) weigh on average 21.7g (SE = 0.21g) and have a wing length of 62.1cm (SE = 0.62cm); females (n = 41) weigh on average 18.6g (SE = 0.22g) and have a wing length of 59.3cm (SE = 0.35cm). For this experiment, we focused on 23 pairs of riverside wrens whose territories we had carefully mapped from April to June 2015 (38 of these birds were previously captured and banded).

Playback stimuli

For the playback stimuli, we used a total of 5 duets, each recorded from different pairs present in the study site. We chose local songs to ensure the stimulus was recognized and provoked a strong response, given that different populations might have different dialects. The stimuli songs were selected from the repertoire of a pair located at least 500 meters apart (more than 3 territories away) to reduce the chance that our focal birds would have

had any prior experience with the particular pair whose songs we broadcast. We recorded these songs using a Sennheiser ME66 directional microphone and a Marantz PMD670 solid-state digital recorder. We selected good quality songs (a high signal-to-noise ratio and no other vocalizations in the background) where the focal birds were singing side by side (less than a meter apart), to ensure that the degree of coordination was relatively consistent across stimuli (Mennill & Vehrencamp, 2008; Mann et al., 2009). To create stereo-duet playbacks (e.g. Mennil, 2006), we duplicated the one-channel recordings and then carefully removed all of the phrases from one sex in one file and all of the phrases from the other sex in a second file using the frequency curser filter function in Syrinx (J. Burt, Seattle, Washington, USA). Afterwards, using Audacity (http://www.audacityteam.org), each file was normalized so that the peak amplitude was 0dB. We created a two-channel stereo sound file containing one channel with male songs and one channel with female songs, thus keeping the exact timing of the original duet. The contribution of each sex was randomly assigned to the left or right channel. The stimuli consisted of 10 bouts of duets, each with 7 song phrases from each sex, separated by 10 seconds of silence, which is consistent with the mean phrases per duet and mean inter-phrase duration previously reported for this species (Mann et al., 2009). Each trial consisted of 5 min of pre-playback period, followed by 3 min of playback, and 5 min of post-playback period.

Playback setup

We used two connected speakers (a Foxpro Fury and a FoxPro SP-55 External Speaker) to broadcast the male and female contributions as a stereo-duet playback (e.g. Mennill, 2006). These two speakers produce standardized outputs (FoxPro Inc., PA, USA) and to our ears they sound equivalent in terms of quality and amplitude (e.g. Templeton et al., 2011, 2013b). We randomized which speaker played the male/female contributions for each trial (with a coin flip), so even if there were differences between speakers they should not

produce any bias in the data. The speakers were set approximately 1-1.5m above the ground and 10m apart to facilitate accurate measures of which speaker each individual was more likely to approach. Riverside wrens commonly sing duets at this height and from this distance (EQG, personal observation). The speakers were placed within pair territories, preferentially along the river for better identification and tracking of individuals. The trials were performed at locations within the territory to avoid neighbour interference during the trials.

Data collection

During the playback trials two observers monitored all playback responses. One observer stayed in the middle of the two speakers to accurately assess approaches to each speaker. The second observer was positioned 10m away to maximize the accuracy of distance measurements while minimizing our overall influence on the birds' approach response. Most of the time both of the focal birds were in sight and easy to track, but in some territories with especially dense vegetation the location of the birds was sometimes estimated from their songs. Because the speakers were 10m apart, whenever an individual was inside the 5m radius of either speaker it was considered to be closer to that speaker than to the other one. During the trials we recorded all vocalizations from the focal individuals and assessed the distance of each bird to both playback speakers and to each other as often as possible and every time any bird moved. Pair members were considered to be in close proximity (as opposed to apart) when they were one meter or less away from each other.

We carried out this experiment on 23 territories. In 22 territories at least one adult member was previously marked (39 colour-banded individuals in total). In the remaining territory where neither of the individuals were banded we distinguished each sex by the songs produced by each bird (Mann et al., 2009). The trials were conducted from the 11th to the

22nd of June 2015 at 0600-0900 h to minimize effects of date and time of day on the behavioural responses to playback. We chose this time period to conduct trials when birds are vocally active before temperature and humidity rise during the day and to avoid any potential confounding effect of the dawn chorus.

Before initiating the trial, we conducted a five-minute pre-playback period to ensure that birds were not provoked by other stimuli (e.g. other territorial intruders) and to obtain baseline data regarding the typical behaviour of pairs (vocal activity and distance between individuals) in the absence of territorial intruders. However, the number of trials in which birds were observed and/or sang during the pre-playback period was not large enough to create baseline values. Therefore, we used data collected during sound recordings from a random sample of 20 pairs made during 2015 and 2016 to determine the vocal activity and distance between pair members in natural contexts, unprovoked by playback.

### Statistical Analyses

In 20 out of 23 territories both adult pair members approached the speakers during the playback. In the remaining three territories only males came within sight (we believe these females did not approach because they were incubating and reluctant to leave their nests). We excluded these three pairs from the analysis. Although juveniles were found in four territories, they never responded to playback—none of them sang nor approached the speakers—nor did their behaviour seem to affect the response of the adults, so we disregard their presence for statistical analyses. Thus, the final sample size for the analyses was 20 pairs.

To determine the acoustic behaviour in response to a simulated intrusion we examined the following variables in each pair: 1) Number of duets sung, 2) number of duets where each

sex is the one to sing the first contribution, 3) number of duets where each sex is the one to stop singing (thus terminating the cycle of the duet), and 4) number of phrases sung by each sex while duetting.

To examine whether pairs sang more duets in response to playback than during the pre-playback period we used a Wilcoxon signed rank test for paired samples. To compare the number of duets where each sex sings the first contribution, the number of duets where each sex stops singing, and the number of phrases sung by each sex in natural and playback contexts we used Wilcoxon signed rank tests for paired samples. To analyse whether the proportion of duets where each sex sings first, the proportion of duets where each sex stops singing, and the proportion of phrases sung by each sex varied between natural and experimental contexts, we used Wilcoxon rank sum tests for unpaired samples.

To determine the physical behaviour during playback we examined the following variables in each pair:

1) Proportion of time pair members spent in close proximity (≤1m), 2) time spent near each speaker (i.e. ≤5m) while pair members were in close proximity, 3) frequency of individuals approaching the same-sex or opposite-sex speaker while pair members were apart, and 4) closest approach distance of each individual to each speaker.

1) To test whether pairs spend more time in close proximity (≤1m) than apart we used a one-sample Wilcoxon signed rank test. We compared the proportion of time in close proximity to the value of 0.5, since this is the proportion that corresponds to pairs spending the same time in close proximity and apart. 2) The time spent on the male versus female speaker while the pair members were in close proximity was analysed using a Wilcoxon signed rank test for paired samples. 3) The frequency of individuals from each sex approaching the same- or opposite-sex speaker when they were apart was analysed using a Fisher's exact

test. Only for this test we used a reduced sample of 14 males and 9 females. This was due to the fact that 10 birds (from five pairs) were never apart (>1m) for the whole duration of the playback, and because we only considered individuals that were within the 5m radius of one or the other speaker and thus showed a clear preference. 4) To compare the closest approach between male and female individuals and between male and female simulated intruders we used generalized estimating equations (gee). This modelling approach was chosen because it accounts for the lack of independence among observations within territories (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). For this analysis, we used bird sex, speaker sex and an interaction term between them as covariates, and modelled closest approach with a gamma distribution that best fitted the error distribution.

We also compared the proportion of times seen in close proximity (≤1m) in natural (unprovoked by playback) and experimental (playback trials) contexts with a Wilcoxon rank sum test for unpaired samples. A within-territory analysis was not possible because several pairs had few or no natural observations during pre-playback. Therefore, for the natural contexts we used recordings of a random sample of pairs recorded without the use of playback (unprovoked). We chose natural recordings that lasted at least 10 minutes to make sure we would sample a full range of behaviours and not just when birds were singing together. Because riverside wrens are very inconspicuous and extremely mobile, on several occasions during the natural recordings we were not able to assess where the individuals were. Therefore, instead of using the percentage of time pairs spent in close proximity or apart during the total time of recordings, every time it was possible to assess if pair mates were in close proximity or apart, it was done so. The proportion of far and close observations was then calculated from the total number for each separate observation within a recording for each distance class (i.e. each time the pair, or an individual became visible again during a recording so proximity could be assessed it was scored as near or far).

285 Statistical analyses were conducted using R 3.1.0 software (R Core Team 2014), using the
286 packages *geepack* and *boot*.
287
288 Ethical Note
289 The University of St. Andrews Animal Welfare and Ethics Committee approved this work.
290 The handling and ringing of birds was done only by those with previous experience. During
291 the captures we attempted to minimize the stress on birds and released them as soon as
292 we had banded them. Birds were followed until we heard them singing again or until we saw
293 them re-joining their partners. All birds were seen and recorded on the following days after
294 capture.
295
296
297 **Results**
298
299 Pairs responded to a simulated territorial intrusion by highly increasing their duetting
300 output (duets per 3 min) from 0.75 ± 0.39 during pre-playback to 6.5 ± 0.58 during playback
301 (Wilcoxon signed rank test, V = 190, $P < 0.001$). Duets comprised 86 ± 5.1% of the total
302 song output throughout playback. Territorial defence elicited riverside wren females to
303 increase the proportion of duets in which they sang the first contribution from 0.2 ± 0.06
304 during natural context to 0.44 ± 0.5 during playback (Wilcoxon rank sum test, W = 103.5, $P$
305 $< 0.01$); and to decrease the proportion of duets in which they stopped singing from 0.84 ±
306 0.4 during natural context to 0.62 ± 0.7 during playback (Wilcoxon rank sum test, W = 289.5,
307 $P = 0.01$). Throughout natural contexts, duets comprised 91 ± 3% of the total song output,
308 males sang more than females the first contribution in duets (V = 196.5, $P < 0.001$), males
309 sang more phrases than females when duetting (V = 164.5, $P < 0.01$), and females stopped

singing in more duets than males (n = 20, V = 2.5, $P < 0.001$). Throughout playback, males and females sang the first contribution in similar numbers of duets and sang similar number of phrases while duetting (V = 104.5, $P = 0.19$; V = 114, $P = 0.22$), but females stopped singing in more duets than males (V = 41, $P < 0.03$).

Pairs were significantly more likely to be in close proximity (≤1m) during a simulated intrusion than during an unprovoked context (Fig. 2, Wilcoxon rank sum test, W = 53, $P < 0.001$). While partners were seen within a meter of one another only 32% of the times during natural contexts, in response to playback, pairs spent on average 81% of the trial in close proximity (SE = 4.1%, median = 89.7%). During playback trials pair members spent significantly more time in close proximity (≤1m) than apart (Wilcoxon signed rank test, n = 20, W = 206, $P < 0.001$), with five pairs never separating more than this distance during the entire playback period, moving together even when moving relatively large distances within their territory. In 14 out of the 20 territories, both individuals arrived simultaneously at the speakers. In the remaining territories, males arrived first but females joined them after less than 20 seconds.

When birds were in close proximity during playback, they spent equal amounts of time at both speakers (Fig. 3, Wilcoxon signed rank test, n = 20, W = 103, $P = 0.95$). On average, pairs spent 53s (SE = 13.2s, median = 32.5s) close to the male speaker (i.e. ≤5m) and 51s (SE = 10.7s, median = 45.5s) close to the female speaker (i.e. ≤5m). During the relatively few time periods when birds were apart, individuals showed a same-sex bias in approach behaviour, with more males (11 out of 14) approaching closer to the male speaker and more females (7 out of 9) approaching closer to the female speaker (Fisher's Exact Test, two-sided, n = 23 $P = 0.01$).

Overall, a male's closest approach distance to the male and female speakers was on average 3.4m (SE = 0.99m, median = 2m) and 5m (SE = 1.06m, median = 3m), respectively. For females, the closest approach distance to the male and female speakers was on average 4.9m (SE = 1.05m, median = 3m) and 4.8m (SE = 1.11m, median = 2.5m), respectively (Fig. 4). No significant statistical differences were found among sexes or among speakers. However, a trend ($P$ = 0.076) existed for the interaction term between sex and speaker suggesting males might approach closer to the male speaker but females did not discriminate.

## Discussion

Riverside wrens primarily responded to simulated pairs of intruders by arriving together and staying in close proximity the majority of the time rather than responding at different times or approaching the speakers separately. Both pair members reacted with equal levels of aggression in their approaches to the two intruders: they were similarly close and spent comparable time next to the male and female speakers. The simulated intrusion elicited individuals to highly increase their duetting output and to show equal levels of vocal participation: pairs coordinated most of their songs to form duets and females sang the first contribution in as many duets and sang as many phrases as males during the territorial defence. Our findings suggest that riverside wrens not only display convergent behaviour during a paired intrusion but also that pair members are more invested in maintaining a cooperative territorial defence rather than performing same-sex specific responses.

The symmetry and intensity of the response in riverside wrens indicate that pair members are highly interested in defending the shared territory and that both individuals in a pair are

willing to actively engage with both intruders. Most previous studies with stereo duet playback have documented duetting pair members approaching male and female simulated individuals with different intensities. For example, eastern whipbirds (*Psophodes olivaceus*) show no coordination during defence and mostly same-sex aggression (Rogers et al., 2007); rufous-and-white wrens' (*Thryophilus rufalbus*) aggression is also biased towards same-sex intruders with females showing a weaker response overall (Mennill & Vehrencamp, 2008); happy wrens (*Pheugopedius felix*) approach closer to the same-sex speaker with none approaching closer to the opposite-sex speaker (Templeton et al., 2011); in black-bellied wrens (*Pheugopedius fasciatoventris*), although males respond strongly to both intruders, females approach closer to same-sex intruders (Logue & Gammon, 2004); and in barred antshrikes (*Thamnophilus doliatus*) males approached much faster and closer to the speakers compared to females, even if each sex showed similar responses to the two intruders (Koloff & Mennill, 2013). To our knowledge, in only three duetting species where distances between partners has been assessed, have equal levels of attention to the two simulated individuals been shown: magpie-larks (*Grallina cyanoleuca*) flew mostly as a 'united pair' towards the speakers and made 93% of their flights together approaching the same speaker (Rogers et al., 2004); stripe-headed sparrows (*Peucaea ruficauda*) reacted with the same intensity in their physical response (Illes & Yunes-Jimenez, 2009); and in yellow napped amazon parrots (*Amazona auropalliata*), pair members showed an equally aggressive response, staying less than 10m apart during playback and approaching speakers mostly together (Dahlin & Wright, 2012).

In species that have size dimorphism, different levels of aggression might be predicted by territory holders because the bigger sex would experience lower costs when confronting any intruders (of either sex), therefore it should be this sex that would be more prone to intersexual territoriality (Logue & Gammon, 2004). However, it has been shown that

coordinated duets are an important signal during territorial encounters (Hall & Magrath, 2007). Perhaps in riverside wrens the weight difference between sexes is not large enough to deter females from confronting intruding males. For them, the benefits of defending their territory and their mate are higher than the potential costs of interacting with larger individuals, especially if they engage in this competitive behaviour side by side with their partner (Hall, Rittenbach, & Vehrencamp, 2015). Considering that this species sings one of the most coordinated duets described so far (Mann et al., 2009) and that acoustic coordination improves when mates are closer (Mennill & Vehrencamp, 2008; Templeton et al., 2013a), it does seem likely that partners might jeopardize that synchrony if they were to confront their intruders separately. Therefore, remaining in close proximity (≤1m) and displaying a joint behaviour during the defence of their territory could be highly important to both pair members in order to show commitment and stability to outsiders through song coordination. One alternative to the cooperative hypothesis is that the pairs remain in close proximity because individuals are preventing their mate from engaging in extra pair copulations (i.e. mate-guarding, Stokes & Williams, 1968). We did not test for the responses to simulated solo intruders, so we cannot reject the possibility that individuals might perform a close joint defence as an attempt to guard the pair bond. In duetting species that have year-round territories and long-term partnerships, the defence of the shared resources and the partnership are tightly connected because acquiring a new mate or territory can both be challenging (Rogers et al., 2004, Hall & Peters, 2008, Logue & Hall, 2014). Riverside wrens share several activities including nest building and parental care (Skutch 2001 and EQG, personal observation), which suggests males and females benefit from maintaining and protecting the pair bond as well as the territory (Hall, 2004, Rogers et al., 2004, Logue & Gammon, 2005).

While pairs spent the vast majority of the trial in close proximity, when they did separate, each individual primarily approached the same-sex speaker. This observation could support the same-sex defence (Logue & Gammon, 2004; Mennill & Vehrencamp, 2008) or the mate-guarding hypothesis. Under same-sex defence, the risks of interacting with a bigger individual are greater for females if their mates do not join them. Hence, when they are apart there is less threat if they follow a division of labour where females confront females while males confront males. Under the mate-guarding hypothesis, individuals seek to advertise their partner's mated status by singing and showing themselves to the same-sex intruder. We found that riverside wrens approach the speakers within a distance close enough to engage in direct contact, suggesting that birds are prepared to physically challenge intruders. Additionally, we did find a trend (albeit not significant) that male riverside wrens approached closer to the male speaker than to the female one, also showing that males are perhaps more invested in confronting other males. The turnover rate in riverside wren territories is actually high (around 50% of the birds either leave the territory or die every season, E. Quirós-Guerrero own data), which shows that mate change is likely so birds must treat same-sex individuals as a strong threat. Divorce entails a cost because it could lead to a loss of the territory or other resources in it or because experience improves the breeding success between mates (Benedict, 2008). Thus, it is not surprising that when riverside wrens are apart, each mate is more likely to engage with an individual endangering their territory ownership as well as their mated status (Logue, 2005; Pärn, Lindström, Sandell, & Amundsen, 2008). Further work investigating the responses of males and females towards single intruders might help elucidate if there are any sexual conflicts within the partnership in this species. Additionally, it would be very interesting to address in the future the effect of varying distances between simulated intruders seeing how close riverside wrens remained in response to this study.

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## Figures

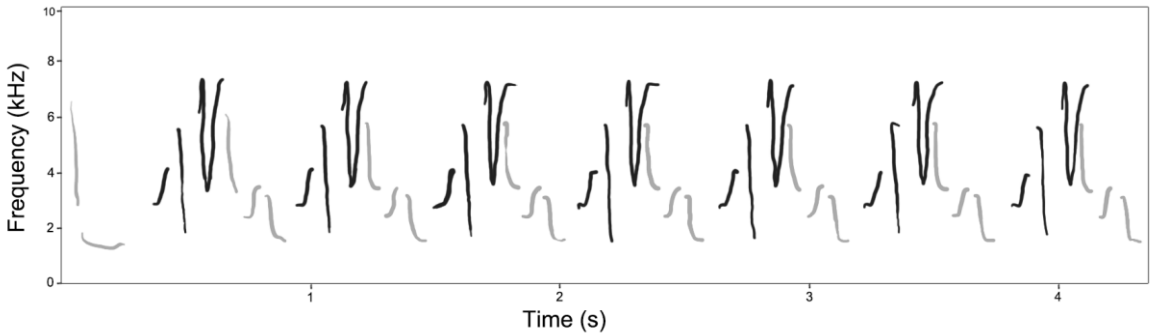
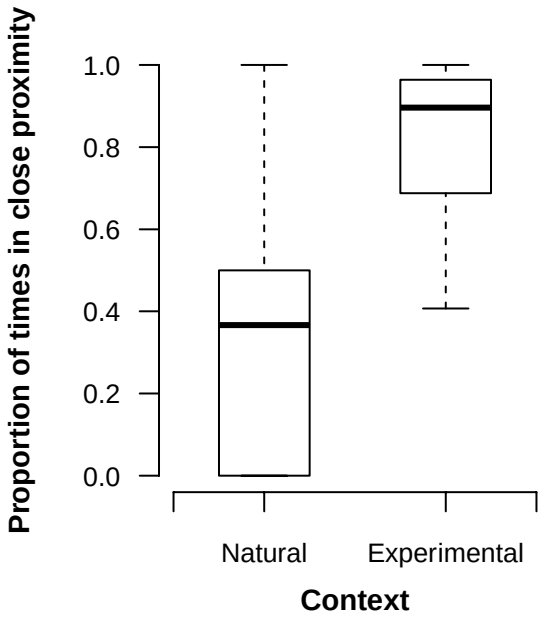



Figure 1. Tracing of a spectrogram illustrating an example of the high coordination in a single riverside wren duet song type. The male contribution is depicted in grey and includes an introductory phrase (I phrase) and the male sex specific phrase (M phrase). The female contribution is depicted in black and includes a female sex specific phrase (F phrase). Pairs have repertoires of approximately 40 of these song types.

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592 Figure 2. Boxplot of proportion of times pairs were seen in close proximity (≤1m) in the

593 natural and experimental contexts across territories.

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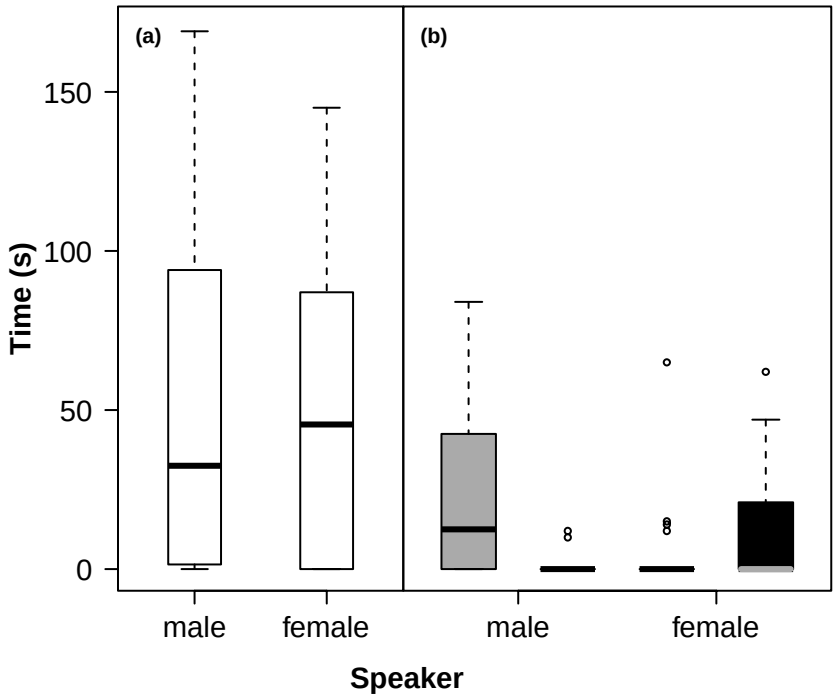


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596 Figure 3. Boxplots of time during trial spent on the male and on the female speaker. The trial
597 lasted approx 180s. a) When in close proximity (≤1m apart), pairs approached both speakers
598 equally. b) When individuals were not in close proximity (<20% of the time), birds were more
599 likely to approach the same sex speakers; male behaviour is represented with the grey
600 boxplots and female behaviour is represented with the black boxplots.

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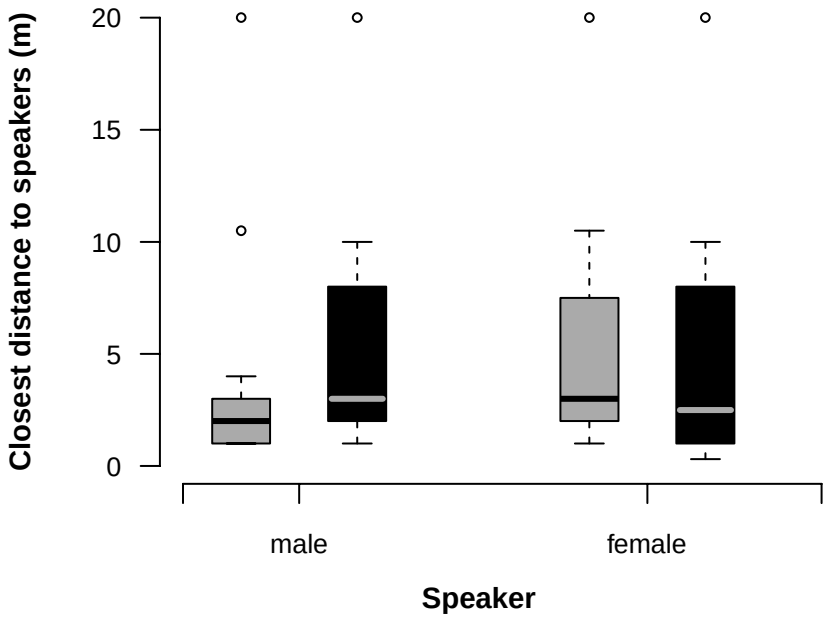


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606 Figure 4. Boxplot of closest approach distance of males and females to the male and female
607 speaker. Male individuals are represented with the grey boxplots; female individuals are
608 represented with the black boxplots.